Abstracts

1. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

Part 7. Experiments on Mice

No. 1. Virulence of Various Acid-fast Bacilli Against Mice

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A series of experiments was carried out on hybrid mice in order to study the virulence of various acid-fast bacilli and BOK (a variant strain of human tubercle bacillus "Kawakami").

Group	I	II	III	IV	V	VI	VII
infected with	0.1mg of "H_2"	0.01mg of "H_2"	0.001mg of "H_2"	0.1mg of "$H_{37}Rv$"	0.1mg of Bovine	0.1mg of BCG	0.01mg of BCG

VIII	IX	X	XI	XII	XIII	XIV
0.001mg of BCG	0.1mg of Timothy	0.01mg of Timothy	0.001mg of Timothy	0.1mg of BOK	0.01mg of BOK	0.001mg of BOK

As shown above, 210 mice, each 13 gm or so in weight, were divided into 14 groups and the bacilli were intravenously injected to them. The animals of each group were sacrificed, two or three at a time, the day after the injection and at the end of each week up to the sixth. Each time macroscopic and microscopic observation of the pathological changes and quantitative culture of the bacilli present in the viscera (lungs, liver and spleen) were carried out.

The results obtained are summarized as follows:

1) The difference of multiplication between human, bovine and other strains of tubelcule bacilli was observed to be most conspicuous in the lungs, and macroscopical and microscopical changes of the lung ran nearly parallel with the number of microbes present in the organ as shown by the colonies developing in the quantitative culture.

2) In the lung, it was observed, the number of human and bovine tubercle bacilli continued to increase for six weeks after the infection, and BCG increased for two weeks and then gradually decreased, while B. timothy and BOK decreased from the outset.

3) In the liver and the spleen, the multiplication of tubercle bacilli were proved to be not so remarkable as in the lung after the infection of human tuercle bacilli and increase and decrease of number of the bacilli showed almost the same tendency at every stage for each bacillus, furthermore, macroscopically, tubercles were not observable at any stage of the observation.

2. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

Part 7. Experiments on Mice
No. 2. Protective Power of Various Bacillar Vaccines, o-Aminophenol Azo-Tuberculin (O. A. Azo-T.) and o-Aminophenol Azo-Tuberculoprotein (O. A. Azo-Tbc-P.) Against Tuberculous Infection

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A series of experiments was carried out in oder to study the defence power of mice pretreated with living, heat-killed and phenol-killed BCG against tuberculous infection.

Each group of mice, which had received intravenous inoculation of these BCG vaccine, was again divided into two subgroups. Animals of subgroup A were challenged with human type tubercle bacilli "H_2" by the same route two weeks after inoculation, while those of subgroup B were challenged four weeks after inoculation, and killed two weeks afterward.

Macroscopical and microscopical examination of the pathological changes of the animals' viscera and quantitative cultivation of the bacilli present in them were carried out.

Further experiments were conducted in which B. timothy, BOK, "H_2" (heat-killed paraffin oil vaccine), O. A. Azo-T. and O. A. Azo-Tbc-P. were employed instead of BCG.

The results obtained are as follows:

1) Living BCG was observed to be most effective against tuberculosis challenge, and phenol-killed BCG, heat-killed paraffin oil "H_2", O. A. Azo-T. and O. A. Azo-Tbc-P. were to be effective only to a small extent.

2) Heat-killed BCG, B. timothy and BOK were observed to give no defence power to mice against tuberculous infection.

3) In order to observe the effect of the pretreatment in mice, it appears advantageous to carry out challenge infection 4 weeks after the pretreatment and to examine the result 2 weeks thereafter.

3. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

Part 7. Experiments on Mice
No. 3. Experiments on Guinea Pigs (Comparison with Experiments on Mice)

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A number of tuberculin-negative, healthy white guinea pigs, each weighing from 200 to 300 gm, were divided into 5 groups and treated as follows:

Group	Pretreated with	Challenged with, four weeks after the pretreatment
I	5 mg living BCG	0.1 mg living "H_2"
II	5 mg heat-killed BCG	〃
III	1 mg O. A. Azo-Tuberculin "H_2"	〃
IV	5 mg living BCG	None
V	None	0.1 mg living "H_2"

The guinea pigs of first three groups were sacrificed 2 and 4 weeks after the challenge infection, and the guinea pigs of the last two groups were used as controls.

The results obtained are as follows:

1) All the animals pretreated became tuberculin positive within reasonable periods.

2) No parallelism was observed between tuberculin allergy and resistance against tuberculous infection.

3) Heat-killed paraffin oil "H_2" was as effective as BCG, but O. A. Azo-Tuberculin "H_2" was less effective than either of them in enhancing the resistance against infection.

Thus, from the data of all the 3 reports it may be noted here that the hybrid mouse is not inferior to the guinea pig as an experimental animal for the study of tuberculosis.

4. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

Part 6. Studies on Tubercle Bacilli Isolated from Sputa Collected from Street Surface

No. 1. Isolation of Tubercle Bacilli from Sputum Found on Street Surface

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Speciment of sputum, 115 in number, collected from the street surface of Hakodate City in November 1953 were examined for tubercle bacilli by the cultivation onto Ogawa's egg-yolk media, and the streptomycin (SM)-resistance of the bacilli isolated was measured by indirect method using Kirchner's fluid media containing SM in different concentrations; 0, 1, 10, 100 and 1,000 γ/ml.

The results obtained are summarized as follows:

1) In 10 out of the 115 sputa, colonies of tubercle bacilli became visible on Ogawa's media in the course of 8 weeks incubation at 37°C.

2) The bacilli cultivated on Ogawa's media were inoculated into Kirchner's media

containing different concentration of SM and three of the cases were found to grow in media containing 100 γ/ml and two others in those containing 10 γ/ml.

5. FORMATION OF TUBERCULIN BY WASHED TUBERCLE BACILLI IN CITRATE SOLUTION

Part 8. Concentration and Purification from Citrate-Tuberculin of the Tuberculin-Active Principle

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The present work is concerned with concentration and purification of the tuberculin-active substance from "citrate-tuberculin", which was obtained by incubating washed tubercle bacilli in a citrate solution.

The tubercle bacillus used was the human strain Aoyama B. A series of flasks (500 ml in capacity), each containing 250 ml of Sauton's medium, was inoculated with Aoyama B and incubated at 37°C for about 40 days, at the end of which period the bacilli in each of the flasks were harvested, washed thrice with 100 ml of sterile distilled water and immersed in 50 ml of sterile 0.1 M neutralized citrate solution, being followed by incubation at 37°C for about 30 hours. At the end of the incubation, the citrate solution separated from the bacilli was pooled and passed through Seitz filter. The citrate-tuberculin thus obtained possessed as active a tuberculin potency as the culture filtrate of the tubercle bacilli grown on Sauton's medium for about ten weeks.

The method which was successfully used in this work for isolating the active substance of citrate-tuberculin was as follows: Ammonium sulfate was added to the solution up to 80 per cent of saturation. The mixture was allowed to stand overnight in the refrigerator, and the precipitate was centrifuged and dissolved in a small amount of 0.1 M Na_2HPO_4, then the insoluble matters were centrifuged off. The clear supernatant was dialysed against distilled water overnight in the refrigerator. To the dialysed solution was added acetic acid until the pH fell to 3.8-4.0. The precipitate (Ac-Fraction) was centrifuged. The supernatant was brought to 30-50 per cent of saturation by addition of solid ammonium sulfate. The precipitate (Am-I-Fraction) was centrifuged. The supernatant was brought to 70-80 per cent of saturation with more ammonium sulfate. The precipitate (Am-II-Fraction) was centrifuged.

The yield of the three fractions from 1620 ml of citrate-tuberculin was Ac-Fraction 86 mg, Am-I-Fraction 34 mg, and Am-II-Fraction 11 mg.

The tuberculin skin tests, which were carried out on tuberculous guinea pigs, revealed: (a) Am-I-Fraction was the most active, the potency being two or more times as active as o-aminophenol azo-tuberculin; (b) both Ac- and Am-II-Fractions were only about one-fifth or one-tenth as active as Am-I-Fraction.

Am-I-Fraction showed strong positive ninhydrin and biuret reactions, but it gave neither Molisch nor diphenylamine reaction.

6. CLINICAL STUDIES IN CHEMOTHERAPY OF TUBERCULOSIS

Part 24. Observation on the Combined Use of Pyrazinamide and Isoniazid in the Treatment of Pulmonary Tuberculosis

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Pyrazinamide-isoniazid has been together used in the treatment of 5 patients with active far advanced pulmonary tuberculosis.

The results obtained were as follows:

1) Pyrazinamide-isoniazid might be effective to the type of lesions above mentioned; all the patients did show symptomatic improvement, and in chest roentgenograms one case actual improvement was seen.

2) The toxic side effects of this drug were so less that they could be negligible in most cases except one accompanied by the manifestations of joint pains. Especially, damage of the liver function was not brought about in any case through the administration of this drug.